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THE POTENTIAL AQUATIC RISK OF WESCODYNE AND ITS ACTIVE INGREDIENTS

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THE POTENTIAL AQUATIC RISK OF WESCODYNE AND ITS ACTIVE INGREDIENTS

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ABSTRACT

Wescodyne, an iodophor compound, is used extensively in Atlantic Region aquaculture operations as a general disinfecting agent for aquaculture gear and fish pens.

Iodophors contain both iodine and a non-ionic surface-active agent that acts as a carrier and solubilizing agent for the iodine in water and in lipids (Budavari *et. al.*, 1996). Iodophors enhance the bactericidal activity of iodine by helping to penetrate lipids. Iodophors also reduce the vapour pressure and odour of iodine, minimize staining, allow a wider dilution with water, and control release rates of free iodine over a specific period of time (Budavari *et. al.*, 1996; Duan and Kessler, 1999). Iodophors are acutely toxic to a broad range of organisms (OEHS, 2003).

Wescodyne contains two individual iodine complexes: polyethoxypolypropoxypolyethoxyethanol-iodine and nonylphenoxypoly(ethyleneoxy) ethanol-iodine (also defined as nonylphenyl ester of polyethylene glycol-iodine complex, which is a nonylphenolic compound). No physical, chemical or toxicological information was found on these two active ingredients. In order to be conservative in the estimation of potential aquatic risk associated with Wecodyne exposure, both the iodine and the nonylphenolic components were assessed. The nonylphenol ester component of Wescodyne was assessed as 4-nonylphenol, branched (referred to in this report as NP). NP was selected as the surrogate nonylphenol compound because the United States Environmental Protection Agency (USEPA) has indicated that this substance best represents nonylphenols (Hellyer, 1991).

Based on an understanding of the physical, chemical and toxicological properties of iodine, NP, and where available, Wescodyne, it appears that components of the iodophor compound are moderately persistent in water and sediment (primarily the NP component) but not expected to bioaccumulate in aquatic organisms.

Use pattern information indicates that at aquaculture facilities Wescodyne is likely entering the marine environment via direct discharge to surface water during disinfection activities. Actual concentrations entering the environment and dispersion rates, however, are not currently known. In order to determine the potential volumes of Wescodyne released to the marine environment from individual facilities and concentrations present in the ambient environment subsequent to release, these values have been calculated based on an understanding of total use by province per year, total use per fish per year, and total use per facility per year (Burridge, 2007; Parker, 2004; Muise and Associates, 2001; Roth, 1998). Other iodophors may also be in use at aquaculture facilities in the Atlantic region (i.e. Mikroklene, Ovadine), but do not contain the nonylphenol component, and because of this, they were not explicitly assessed in this study.

Based on these preliminary calculations using limited data, and using the most conservative literature-based ecotoxicity values involving the most sensitive species tested, Wescodyne may be entering the environment in concentrations that may pose unacceptable risks to marine aquatic receptors in Atlantic Canada.

The results of this study indicate that more information on use pattern, discharge concentrations and rates, and dilution rates are required in order to better characterize concentrations of Wescodyne in the aquatic environment that may be available for exposure by aquatic receptors.

RÉSUMÉ

Le Wescodyne, un composé iodophore, est grandement utilisé dans les exploitations aquicoles de la région de l'Atlantique comme agent stérilisant général pour l'équipement aquicole et les compartiments à poisson.

Les iodophores contiennent de l'iode et un agent de surface non-ionique qui agit comme porteur et agent de solubilisation pour l'iode dans l'eau et dans les lipides (Budavari et coll., 1996). Les iodophores améliorent l'activité bactéricide de l'iode en facilitant la pénétration des lipides. Les iodophores réduisent également la pression de vapeur et l'odeur de l'iode, minimisent les taches, permettent une plus grande dilution avec l'eau et contrôlent les taux d'émission d'iode libre sur une période de temps spécifique (Budavari et coll., 1996; Duan et Kessler, 1999). Les iodophores sont un composé toxique à toxicité aiguë pour un large éventail d'organismes vivants (Services de santé au travail et d'hygiène du milieu, 2003).

Le Wescodyne contient deux iodes individuels complexes : l'iode du polyéthoxypolypropoxypolyéthoxyéthanol et l'iode de nonylphenoxypolyéthoxyéthanol (également défini comme éther mono(nonylphénylique) du polyéthylène glycol composé avec l'iode, qui est un composé nonylphénolique). Aucune information physique, chimique ou toxicologique n'a été découverte sur ces deux ingrédients actifs. Afin d'avoir une estimation conservatrice des risques aquatiques possibles liés à l'exposition au Wescodyne, on a évalué l'iode et les composés nonylphénoliques. Le composé éther de nonylphénol du Wescodyne a été évalué comme 4-nonylphénol, ramifié (désigné dans le présent rapport sous le nom de NP). On a choisi le NP comme composé auxiliaire de nonylphénol puisque la United States Environmental Protection Agency (USEPA) a indiqué que cette substance représente le mieux les nonylphénols (Hellyer, 1991).

Selon la compréhension des propriétés physiques, chimiques et toxicologiques de l'iode, du NP et du Wescodyne, lorsque fournie, il semble que les composants du composé iodophore soient modérément persistants dans l'eau et dans les sédiments (principalement le composant NP), mais on ne croit pas qu'ils soient bioaccumulables dans les organismes aquatiques.

Les données sur la méthode d'utilisation indiquent que dans des installations aquicoles, le Wescodyne entre probablement dans le milieu marin au cours du rejet direct de la substance dans les eaux de surface à la suite des activités de désinfection. Toutefois, les concentrations réelles dans l'environnement ainsi que les taux de diffusion sont actuellement inconnus. Afin de déterminer les volumes possibles rejetés dans le milieu marin par chaque installation et les concentrations présentes dans le milieu ambiant suite aux rejets, on a calculé ces valeurs en fonction de la compréhension de l'utilisation totale par province par année, de l'utilisation totale par poisson par année et de l'utilisation totale par installation par année (Parker, 2004; Muise et associés, 2001; Roth, 1998).

D'après ces calculs préliminaires effectués à l'aide de données limitées, et de valeurs d'écotoxicité basées sur la documentation les plus conservatives, touchant les espèces testées les plus sensibles, le Wescodyne entre probablement dans le milieu par des concentrations qui peuvent présenter des risques inacceptables pour les récepteurs aquatiques marins dans le Canada atlantique.

Les résultats de cette étude permettent d'indiquer que des données supplémentaires sur la méthode d'utilisation, les concentrations et les taux de rejet ainsi que les taux de dilution sont nécessaires afin de mieux caractériser les concentrations de Wescodyne dans le milieu aquatique susceptible d'exposition aux récepteurs aquatiques.

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1.0 PHYSICAL AND CHEMICAL PROPERTIES

Wescodyne is an iodophor compound, which contains 1.6% titratable iodine as its active ingredient (in complexes polyethoxypolypropoxypolyethoxyethanol-iodine [9.1%] and nonylphenoxypoly(ethyleneoxy) ethanol-iodine [8.74%]) (Cantox, 2001). Nonylphenoxypoly(ethyleneoxy)ethanol-iodine is also known as nonylphenyl ester of polyethylene glycol-iodine complex, and is a nonylphenolic compound. Iodophors contain both iodine and a non-ionic surface-active agent that acts as a carrier and solubilizing agent for the iodine in water and in lipids (Budavari *et. al.*, 1996). Iodophors enhance the microbial activity of iodine by helping to penetrate lipids. Iodophors also reduce the vapour pressure and odor of iodine, minimize staining, allow a wider dilution with water, and control release rates of free iodine over a specific period of time (Budavari *et. al.*, 1996; Duan and Kessler, 1999). As a pesticide, iodophors release free molecular iodine which enters micro-organisms and binds with cellular components, potentially blocking or hindering hydrogen bonding and disrupting protein synthesis at concentrations ranging from 30-50 parts per million of free iodine (OEHS, 2003). Iodophors are acutely toxic to a broad range of organisms (OEHS, 2003). Iodophors are highly reactive, have a low toxicity to fish eggs, and are not affected by water hardness (Amend and Pietsch, 1972; OEHS, 2003). In addition, iodophors are more effective at lower water temperatures than many other antimicrobials (Amend and Pietsch, 1972). They also have the additional advantage of being amber in colour, and as the colour fades, the concentration of iodine decreases, enabling users to determine when the solution is no longer effective (Amend and Pietsch, 1972). A 1:150 iodine solution will turn water dark brown (Alberta Agriculture, Food and Rural Development [AAFRD], 2000).

Iodophors are affected, however, by pH, with higher toxicity at lower pH levels (i.e. <6.0) and lower toxicity at higher pH levels (i.e. >8.0) (USDA, 2002; Alderman, 1984; Amend and Pietsch, 1972). At a pH of 6 or less, the toxicity for eggs increases, and at a pH or 8 or more, the antiseptic efficacy decreases (USDA, 2002). For example, in one experiment conducted by Amend and Pietsch (1972), there was approximately a four-fold difference in virucidal activity between pH 6.0 and 8.6. At pH 6.0, the infectious hematopoietic necrosis (IHN) virus was completely destroyed at 4 ppm iodine (Amend and Pietsch, 1972). At pH 8.6, however, 16 ppm iodine was needed to produce the same effect (Amend and Pietsch, 1972). According to Amend and Pietsch (1972), at pH values above 8.0, the biologically activated hydrated iodine cation is converted to inactive iodate, and because of this, a neutral or slightly acidic solution is required to ensure the effectiveness of disinfection. At a pH lower than 6.5, sodium bicarbonate should be added to the solution to increase its alkalinity (Department for Environment, Food and Rural Affairs, National Assembly for Wales, 2002). Typical seawater pH varies between 7.7 in deep water to 8.2 in shallow water (USDA, 2002), indicating that a greater volume of product would be needed to effectively disinfect in salt water than in fresh water.

No physical, chemical or toxicological information was found on the two individual iodine complexes of polyethoxypolypropoxypolyethoxyethanol-iodine and nonylphenoxypoly(ethyleneoxy)ethanol-iodine. In order to be conservative in the estimation of potential aquatic risk associated with Wecodyne exposure, the nonylphenol component of Wescodyne (nonylphenoxypoly(ethyleneoxy)ethanol-iodine) was assessed as a 4-nonylphenol, branched (referred to in this report as NP). NP was selected as the surrogate nonylphenol compound because the United States Environmental Protection Agency (USEPA) has indicated that this substance best represents nonylphenols (Hellyer, 1991). Toxicological information was identified for Wescodyne, iodine and also NP (see Table B.1 in Appendix B for a summary table of toxicity results) (Environment Canada, 2001b; Amend, 1974; Tooby *et. al.*, 1975; Wright and Snow, 1975; Alderman, 1984). Physical and chemical properties of Wescodyne, iodine (I_2) and NP reported in the literature are presented in Table 1.

Table 1 Physical and Chemical Properties of Wescodyne, Iodine (I_2) and NP

Property	Wescodyne Value	Reference	Iodine Value	Reference	NP Value	Reference
CAS RN	8050-84-8	West Penetone, 2001	7553-56-2	Cantox, 2001	84852-15-3	Hellyer,1991
Chemical formula	1.6% iodine in complexes: Polyethoxypolypropoxy polyethoxyethanol-iodine (9.1%) (CAS RN 26614-87-8) Nonylphenoxypoly(ethyleneoxy) ethanol-iodine (8.74%) (CAS RN 11096-42-7)	Cantox, 2001	I_2	Cantox, 2001	$C_{15}H_{24}O_1$	USEPA, 2000
Molecular weight (g/mol)	Not identified		253.81	Cantox, 2001, National Library of Medicine, 2007	220.36	USEPA, 2000
Physical state	Dark brown viscous liquid	West Penetone, 2001	Violet solid	Cantox, 2001	Colourless to pale liquid at room temperature	Reed, 1978; OECD, 1997
Melting point (°C)	Not identified		113.7	Lide, 2006	-8	OECD, 1997
Boiling point (°C)	100	Johnson Diversey, 2003	184.4	Lide, 2006	295-320	Reed, 1978; OECD, 1997
Vapour pressure (mm Hg [25°C])	Not applicable	West Penetone, 2001	0.233 0.3 @ 20°C	USEPA, 2000 NJDHSS, 2001	9.42e-05 (experimental database)	USEPA, 2000
Water solubility (mg/L)	Completely	West Penetone, 2001	330 @ 25°C solubility increases with increasing temperature	Clayton and Clayton, 1994	3-9 for NP and NPEs	Ahel and Giger, 1993a
Log K_{ow}	Not identified		2.49 1.86	Hansch *et. al.*, 1995 USEPA, 2000	4.17-4.48 5.92	Ahel and Giger, 1993b USEPA, 2000
Log K_{oc}	Not identified		1.155	USEPA, 2000	4.708	USEPA, 2000
Henry's Law constant at 25°C (atm-m^3/mol)	Not identified		2.45e-2 7.27e-5	USEPA, 2000 (group method) USEPA, 2000 (VP/WSol)	4.3e-06 (experimental database)	USEPA, 2000
BCF	Not identified		-0.46 5.38	Cantox, 2003 USEPA, 2000	7159 1440 (zebra fish viscera) 245-380 (fathead minnows) 0.9-741 (different fish species)	USEPA, 2000 Huang *et. al.*, 2007 Huang *et. al.*, 2007 Huang *et. al.*, 2007

1.1 Natural Production

Wescodyne and nonylphenols are anthropogenic substances and therefore are not found naturally in the environment (Environment Canada, 2001b).

Iodine occurs naturally in sea water, in both organic and inorganic forms. Organic forms of iodine are any in which the iodine atom is covalently attached to a carbon atom, such as methyl iodide (CH_3I) (Holmes-Farley, 2003). In some coastal areas, organic forms can comprise up to 40% of the total iodine (Holmes-Farley, 2003). All of the various forms can be inter-converted in the oceans by phytoplankton, marine algae, or through abiotic transformations (Holmes-Farley, 2003).

Approximately two-thirds of the inorganic iodine in sea surface water is in the form of iodate (IO^-_3), and one third is in the form iodide (I^-), although there is substantial variation in those ratios (Tian and Nicholas, 1995). The relative concentration of iodide decreases with ocean depth, with non-detectable concentrations a few hundred metres below the surface, with all the inorganic iodine in the form of iodate (Tian and Nicholas, 1995). Total dissolved iodine (organic and inorganic) follows a nutrient profile in the ocean, depleted by biological assimilation at the surface, increasing to a maximum value a few hundred metres below the surface and then falling slightly to a nearly constant value throughout the rest of the water column (Tian and Nicholas, 1995). Iodide and iodate are less acutely toxic to Rainbow trout than iodine, with LC_{50} values (lethal concentration wherein 50% of a test population dies in a laboratory study) of greater than 200 and 850 mg/L, respectively (Holmes-Farley, 2003). The concentration of total iodine in the ocean was reported to be 0.06 mg/L (Tian and Nicholas, 1995).

1.2 Manufacture and Uses

In Canada, two companies were identified as producing Wescodyne: West Penetone Inc. of Anjou, Quebec; and Johnson Diversey Canada Inc. of Oakville, Ontario. In addition, in one study of the efficacy of common disinfectants on the infectivity of *Cryptosporidium parvum* oocysts in a cell culture (Weir *et. al.*, 2002), Wescodyne was obtained from West Chemical Products of Canada, Montreal, PQ. Therefore, in addition to those companies reporting manufacturing Wescodyne in Canada, other companies/ distributors may also import Wescodyne from manufacturers outside of Canada. Wescodyne is sold by veterinary suppliers, and since it may be produced in Canada or imported, total sales data for aquaculture use in Atlantic Canada would be difficult to calculate.

Wescodyne is used primarily as a disinfectant in the aquaculture, agricultural, medical, and food service industries. Wescodyne was previously but is no longer a registered pesticide with the Pest Management Regulatory Agency, under the *Pest Control Products Act*, and is currently regulated by Health Canada and the Canadian Food Inspection Agency (CFIA) under the *Food and Drug Act* (R.S. 1985, c. F-27) (Health Canada. 2004; CFIA, 2003). According to the product information, Wescodyne may be used as a disinfectant for barns and food premises (Health Canada, 2004). According to the CFIA, Wescodyne is intended to be applied in a manner that is in keeping with label requirements (CFIA, 2003). Wescodyne is not specifically registered or approved for use in aquaculture operations in Canada.

In aquaculture, Wescodyne is commonly used in North America and Western Europe to disinfect fish eggs (especially salmonid species) and aquaculture equipment (Kouril *et. al.*, 1998). In Atlantic Canada, it has been primarily used for surface disinfection of employee hands, feet, and equipment including dip nets, diving equipment, tanks, boots, gloves, raingear, mortality containers, platforms and decking at salmon and steelhead trout aquaculture facilities (Muise and Associates, 2001). It is used throughout the year, in marine and fresh water environments and is either applied by dipping equipment into the solution or by spraying surfaces with the solution (Muise and Associates, 2001). Under normal

use for surface disinfection (dip or spray), iodine products can escape to the environment either by dissipation into the air or dilution into the surrounding water (Muise and Associates, 2001).

Based on a study conducted by Roth (1998), in 1997, the annual usage of iodophor compounds in aquaculture (no trade names identified) in New Brunswick was 10,903 L (2728 L for disinfecting fish eggs and 8175 L for equipment), and in Nova Scotia was 2590 L (887 L for eggs and 1713 L for equipment). A recent report of chemical sales for Alpha Chemicals (2006) indicated that one aquaculture facility in NB purchased 768 L of Wescodyne in 2006 (Alpha Chemicals, 2006). Annual purchase of iodophors by aquaculture facilities have thus ranged from 0 to ~800 L, with some relatively large facilities having reported to only using 5 L (Alpha Chemicals 2006; Roth, 1998).

In 1999, the maximum identified gross amount of Wescodyne active ingredient (iodine) used in either Nova Scotia or New Brunswick per fish was 4.1 mg (4.1 g/1000 fish) in the fresh water environment, and 21.3 mg/kg of fish in the marine environment (quantities used were reported differently for fresh water and marine environments) (Muise and Associates, 2001). No specific information was provided by Muise and Associates (2001) on how much, and/or when Wescodyne is used to disinfect nets, diving equipment, fish containers, or platforms/decking.

Increased use of disinfectants at aquaculture operations (including fish eggs, nets, boats, and other equipment that has been in contact with the fish and/or surrounding water) have been as a result of increased incidences of diseases in fish stocks, in particular, the proliferation of Infectious Salmon Anemia (ISA) in aquaculture sites in New Brunswick (first identified in NB in 1996) (Cipriano, 2002).

1.3 Use Restrictions in Aquaculture Facilities in Atlantic Canada

As of 2004, the Brunswick Department of Agriculture, Fisheries and Aquaculture (NBDAFA) (now known as the New Brunswick Department of Agriculture and Aquaculture or NBDAA) had been assessing options for disinfecting aquaculture equipment to protect against ISA, including spraying equipment with a solution of 2000 mg/L iodophor disinfectant. The NBDAA has prepared guidelines for cleaning and disinfection to control the spread of ISA. These guidelines state that iodophor compounds can only be used on-shore and should be used at a concentration of between 100-200 mg/L for a minimum duration of 10 minutes for effective disinfection (NBDAFA, 2003). Follow-up with the NBDAA in 2007 indicated that there have been no new provincial guidelines or standards developed related to the disinfection of aquaculture equipment since 2004.

The New Brunswick Department of the Environment (NBDE) regulates the discharge of wastes released by marine finfish aquaculture sites and requires that all wastes (with no explicit reference to waste disinfectants) generated must be disposed of in a manner approved by the Minister or in a manner acceptable to an inspector (Parker, 2004). Currently the only acceptable manner for disposal of wastes generated by net washing activities requires washing lightly biofouled nets on-site within lease boundaries, within the intertidal zone below the high-water line, or at an approved land-based facility. Net washing off-site in the marine environment is not a manner approved by the Minister or acceptable to an inspector.

The Nova Scotia Department of Agriculture and Fisheries (formerly the Department of Fisheries and Aquaculture) prepared a work procedure for the use of disinfectants and aquaculture sites (Nova Scotia Department of Fisheries and Aquaculture, 2000). The work procedure defines the level of personal protective equipment required by users and that application rates are to be based on the product label recommendations. For Wescodyne, the product label does not specify use in aquatic environments. In addition, the work procedure contains no discussion of location(s) for the use or disposal of disinfectants.

Environment Canada, Pacific and Yukon Region, in conjunction with Fisheries and Oceans Canada, is currently preparing draft guidelines for the use and disposal of specific substances at aquaculture sites

(including bloodwater, dead fish, disinfectants and substances used for net cleaning). For disinfectants, the draft guidelines recommend that their use be minimized, their application in the immediate vicinity of fish bearing waters be avoided, and any wastes be collected and disposed of at a provincially approved on-land waste management area. As of 2007, these Guidelines had not been published. In Atlantic Canada, currently Wescodyne is being used in the immediate vicinity of fish bearing water and wastes are being discharged directly to the environment and are not being collected and disposed of on-land.

2.0 ENVIRONMENTAL FATE AND EFFECTS

As shown in Table 1, limited physical or chemical information for Wescodyne or its nonylphenolic component (nonylphenoxypoly(ethyleneoxy)ethanol-iodine) was identified in the literature. Without this information, an assessment of their environmental fate is difficult. More data were identified for I_2, the active ingredient, and NP, a surrogate compound intended to provide information on the nonylphenolic component of Wescodyne. Due to the paucity of physical and chemical information related to Wescodyne, the data for I_2 and NP were used in order to determine the environmental fate of Wescodyne. Where available, experimental physical/chemical information for I_2 and NP were utilized to predict the environmental fate of Wescodyne. Where this information was not available, it was modeled using EPIWIN v. 3.10 (Estimation Program Interface Suite for Windows). EPIWIN is a physical/chemical property and environmental fate estimation model developed by the USEPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC) (USEPA, 2000). Using basic information about a substance, EPIWIN calculates the boiling point, melting point, vapour pressure, water solubility, octanol-water partition coefficient (K_{ow}), Henry's Law Constant, biodegradation potential, soil adsorption coefficient (K_{oc}), bioconcentration factor (BCF) (the ratio of the concentration of a substance in an organism to the concentration in water), volatilization half-life from water, and level III fugacity modeling to predict partitioning to air, water, soil and sediment.

The experimental data and/or EPIWIN results for I_2 indicate the following:

- It is a solid at typical ambient temperatures and will volatilize to air if released to water or soil;
- It is very soluble in water and is likely to remain dissolved in the water column and not expected to sorb to suspended organic matter;
- It has a low potential to bioaccumulate in aquatic receptors (based on the *Persistence and Bioaccumulation Regulations* under CEPA, 1999 [Government of Canada, 2000]);
- If released to soil it would be very mobile and would likely migrate to surface water bodies via surface runoff or percolate through soil to the groundwater table;
- Transformation half-life values indicate that I_2 is not persistent in soil or water (based on the *Persistence and Bioaccumulation Regulations* under CEPA, 1999 [Government of Canada, 2000]);
- Based on an Environment Canada study of one iodophor disinfectant (Mikroklene), iodine dissociated quite quickly and free iodine had disappeared within 12 hours (Parker, 2003); and
- Level III fugacity modeling indicates that if released directly to water, 20% would partition to air and 80% would remain in the water (Mackay and Shui, 2000 as calculated by USEPA, 2000).

The experimental data and/or EPIWIN results for NP indicate the following:

- It is a liquid at room temperature and may volatilize to air if released to water or soil;
- It is soluble in water and may be present in the dissolved state or sorbed to suspended organic matter;
- NP may have a low to moderate bioaccumulation potential in aquatic organisms (based on the *Persistence and Bioaccumulation Regulations* under CEPA, 1999 [Government of Canada, 2000]);

- If released to soil it would be immobile and would not be expected to leach from soil to groundwater;
- It is predicted to be persistent in water and sediment (based on the *Persistence and Bioaccumulation Regulations* under CEPA, 1999 [Government of Canada, 2000]);
- Level III fugacity modeling indicates that if released directly to water, 12.4% would remain in the water and 87.6% would partition to sediment (Mackay and Shui, 2000 as calculated by USEPA, 2000).

2.1 Environmental Effects

The following section summarizes the ecotoxicological studies identified in the literature for Wescodyne and I_2 with the lowest acute and/or chronic aquatic toxicity to fish and/or *Daphnia magna*. Acute toxicity tests are of a short duration relative to the lifespan of the test organism and can be used to assess lethal and sub-lethal adverse effects. Chronic toxicity tests span a significant portion of the lifespan of the test organism and are used to assess long-term effects related to changes in metabolism, growth, reproduction or survivability. Results of toxicity studies are shown in Tables B.1 and B.2 in Appendix B.

2.1.1 Wescodyne

Wescodyne appears to be effective at controlling surface bacteria and viruses on fish eggs (trout, largemouth bass, and salmon). The prescribed concentrations range from 25 mg/L (as I_2) for several hours to 50 to100 mg/L for 10 to 30 minutes. (Department for Environment, Food and Rural Affairs, National Assembly for Wales, 2002; USDA, 2002; AAFRD, 2000; Alderman, 1984; Wright and Snow, 1975; Amend, 1974; Gerard, 1972). At lower concentrations, reinfestation of the undesired microorganisms occurred, and at higher concentrations (>200 mg/L), Wescodyne was acutely toxic to eyed fish ova. See Table B.2 in Appendix B for the disinfection capability of various free iodine concentrations.

Tooby *et. al.* (1975) conducted 24-, 48-, and 96-h fresh water flow-through toxicity tests of two components of Wescodyne, the I_2 and the non-ionic surfactant, to Harlequin fish (*Rasbora hetermorpha*), and extrapolated the results to derive three month LC_{50} values. The Harlequin fish was selected due to its size and because it has a sensitivity to most chemicals that is similar to the sensitivity demonstrated by the Rainbow trout (*Oncorhynchus mykiss*) (Tooby *et. al.*, 1975). LC_{50} values were derived for I_2 at pH 6.1 and for the non-ionic surfactant at pH 7.0, 8.0 and 8.1 for 24, 48, and 96 hours and extrapolated for three months. LC_{10}s were also calculated for I_2 and non-ionic surfactants for the same pH ranges, however, LC_{10}s were only calculated for 24 and 48 hours for I_2 and for 24, 48 and 96 hours for non-ionic surfactants. See Table B.1 for complete results.

For the non-ionic surfactant, the lowest acute toxicity value was the 96-h LC_{50} which ranged from 15.2 mg/L at pH 7.0 to 52.7 mg/L at pH 8.1. The extrapolated three-month LC_{50} ranged from 9.9 mg/L at pH 7.0 to 29.8 mg/L at pH 8.1. The lowest LC_{10} value ranged from 9.9 mg/L at pH 7.0 to 21.1 mg/L at pH 8.0.

2.1.2 Iodine (I_2)

Laverock *et. al.* (1995) conducted acute (96-h) static toxicity testing of I_2, IO^-_3, and I^- using *Daphnia magna* and Rainbow trout (*Oncorhynchus mykiss*). Both species were most sensitive to I_2 and much less sensitive to IO^-_3, and I^-. The 96-h LC_{50} for *Daphnia magna* exposed to I_2 was determined to be 0.59 mg/L and the 96-h LC_{50} for Rainbow trout exposed to I_2 was 1.67 mg/L.

Tooby *et. al.* (1975) assessed the lethality of I_2 and the non-ionic surfactant to Harlequin fish. For I_2, the lowest acute toxicity value was the 96-h LC_{50} of 22.8 mg/L, and the three-month LC_{50} was 19.9 mg/L. The lowest LC_{10} value for I_2 was the 48-h LC_{10} of 13.5 mg/L.

2.1.3 Free Iodine Compared to Wescodyne and its Non-Ionic Surfactant

In comparing the toxicity associated with free iodine from Laverock *et. al.* (1995) and free iodine released as part of Wescodyne from Tooby *et. al.* (1975), it appears that I_2, when released from the commercial product Wescodyne, is less toxic to aquatic receptors than the I_2 itself (Rainbow trout 96-h LC50 of 1.67 mg/L at pH 7.7± 0.3 in Laverock *et. al.* [1995] and 22.8 mg/L at pH 6.1 in Tooby *et. al.* [1975]).

When comparing the toxicity of I_2 to the non-ionic surfactant of Wescodyne (from Tooby *et. al.*,1975), the non-ionic surfactant was more toxic than the I_2 portion of the product, particularly at lower pH levels (96-h LC_{50} of between 15.2 mg/L at pH 7.0 to 52.7 mg/L at pH 8.1 for the non-ionic surfactant, and 22.8 mg/L at pH of 6.1 for the I_2 component). In terms of extrapolated three-month toxicity, the LC_{50} for the non-ionic surfactant ranged from 9.9 mg/L at pH 7.0 to 29.8 mg/L at pH 8.1, and the LC_{50} for I_2 was 19.9 mg/L at pH 6.1 (Tooby *et. al.*, 1975).

2.1.4 Nonylphenol (NP)

As indicated previously, the NP selected as the surrogate for nonylphenoxypoly(ethyleneoxy)ethanol-iodine was 4-nonylphenol, branched (Chemical Abstracts Service Registry Number or CAS RN 84852-15-3), which, according to the USEPA, best represents NPs (Hellyer, 1991). In a comparison of physical, chemical and toxicological properties of NP and several nonylphenol ethoxylates (NPEs), NP is more volatile, more persistent in water and sediment than NPEs, and is also more acutely toxic to aquatic receptors (Environment Canada, 2001b). The toxicity of NPEs decreases with increasing ethoxylate chain length (Environment Canada, 2001b). Both NP and NPEs are expected to have a low to moderate bioaccumulation potential in aquatic receptors (Environment Canada, 2001b). In terms of endocrine disruption potential, NP was slightly more potent than the NPEs studied in inducing vitellogenin in trout hepatocytes in one study, and the addition of ethoxylate units to NPEs further reduced the potency of NPEs (Environment Canada, 2001b). For NP, the threshold for vitellogenin induction in fish was 0.01 mg/L, and the threshold for expression of intersex (ova-testes) in Japanese medaka (*Oryzias latipes*) was <0.05 mg/L (Schwaiger *et. al.*, 2002; Environment Canada, 2001b).

NP is acutely toxic to aquatic organisms, with a lowest published acute toxicity value (96-h LC_{50}) of 0.017 mg/L for the marine fish Winter flounder (*Pleuronectes americanus*) (Lussier *et. al.*, 1996). Acute toxicity studies for fresh water and marine fish species have been conducted with 96-h LC_{50} values ranging from 0.017 to 1.4 mg/L, with most values ranging from 0.1 to 0.3 mg/L (Environment Canada, 2001b). For invertebrates, acute LC_{50} values ranged from 0.02 to 3 mg/L, and acute LC_{50} values for algae ranged from 0.027 to 2.5 mg/L (Environment Canada, 2001b). Chronic toxicity values examining growth and reproduction (no observable effect concentrations or NOECs) were as low as 0.006 mg/L in fish and 0.0039 mg/L in invertebrates (Environment Canada, 2001b).

2.2 Critical Toxicity Values

Given that the acute and chronic toxicity of Wescodyne as a mixture is generally lower to aquatic receptors (based on the identified studies which are primarily fresh water) than free iodine or NP separately, it appears that some component of the mixture is responsible for the reduction in toxicity of both I_2 and the NP component. Thus, in order of relevance for use in this assessment, acute and chronic toxicity studies using Wescodyne were considered the most relevant, followed by acute and chronic studies for I_2 and NP. Based on a literature search, it appears that only acute and chronic toxicity testing of Wescodyne has been conducted to date on aquatic organisms (i.e. no studies for endocrine disruption potential). Since NP acts as an endocrine disruptor (Stephanou and Giger, 1982; Jobling *et. al.*, 1996; 1998; Gray and Metcalf, 1997; Comber *et. al.*, 1993; Yadetie and Male, 2002; Hemmer *et. al.*, 2002 – as cited in Huang *et. al.*, 2007), endocrine disruption studies for NP were used to assess the potential for Wescodyne to cause endocrine disruption.

Therefore, for the free iodine in Wescodyne, the critical toxicity value selected was the extrapolated three-month LC_{50} of 9.9 mg/L (Tooby *et. al.*, 1975) for the Harlequin fish. By applying a factor of 10 to convert an LC_{50} to a NOEC, the resultant acceptable exposure concentration of I_2 would be 0.99 mg/L (Kenaga, 1982; Mayer *et. al.*, 1986).

For NP, the critical toxicity value selected was the lowest NOEC identified in the literature (from Ward and Boeri [1991]), with a NOEC for mysid shrimp of 0.0039 mg/L (growth). This concentration was lower than the estimated threshold concentration for endocrine disruption of 0.01 mg/L for NPs (Environment Canada, 2001b; Schwaiger et. al., 2002).

2.3 Environmental Concentrations

Based on its use pattern as a disinfectant at aquaculture sites, Wescodyne is likely entering the aquatic environment via direct discharge to surface water during disinfection activities (Muise and Associates, 2001). Therefore, because of the solubility of its components (I_2, and to a lesser extent, the NP component), Wescodyne, if discharged, would disperse into the larger ambient aquatic environment. The actual concentrations in the environment, and dispersion rates are not currently known. However, the volumes released can be estimated based on a knowledge of sales and discussions with individual users.

The greatest volume of Wescodyne was reported to have been used at marine aquaculture sites in Atlantic Canada rather than at fresh water aquaculture sites, therefore, the estimation of environmental concentrations will be focussed on the marine environment. The greatest volume of Wescodyne was reported to have been used at salmon aquaculture sites (in comparison to Steelhead trout facilities), therefore the estimation of Wescodyne use at aquaculture facilities focussed on salmon aquaculture facilities rather than Steelhead trout facilities. Finally, there are currently a greater number of salmon aquaculture facilities in Atlantic Canada than Steelhead trout facilities. Therefore, in order to be conservative in the determination of environmental concentrations of Wescodyne entering the marine environment at aquaculture facilities in Atlantic Canada, Wescodyne use at marine salmon aquaculture facilities was the focus of this study.

According to Parker (2004), boats, all equipment on board, diving gear, and workers feet and hands, are disinfected following every visit to each aquaculture site, either by spray, or by pouring over or soaking in, a solution of Wescodyne. Particularly in areas where ISA may be present or in areas where facilities are trying to control the spread of ISA, discharge volumes can range from a few drops to several litres of product at any one time (Parker, 2004). According to Parker (2004), it is possible that an aquaculture facility may use up to 95 litres of a mixture of Wescodyne and water to disinfect a boat and equipment following a site visit, which is expected to be no more than once daily (Parker, 2004). In one area of New Brunswick, there are approximately 95 aquaculture farms, and there is the potential that they all may be following the same decontamination procedures and releasing Wescodyne into the marine environment on a regular basis (Parker, 2004). The area that may receive the highest concentration of Wescodyne releases may be at the dock or to and/or from the aquaculture cages, where many decontamination procedures occur (Parker, 2004).

In a study of pesticide dispersion with dye tracers in six areas in the Lower Bay of Fundy, Ernst *et. al.* (2001) determined that following release, typical dilution rates varied between 1/200 to 1/3000 the pre-release concentration after two to five hours, and that distances traveled by the plume varied between 900 and 3000 m during that time.

The following sections provide four separate calculations for determining potential concentrations of free iodine and NP from Wescodyne that may be entering the marine environment from aquaculture activities and dilution rates based on those in the Lower Bay of Fundy (Ernst *et. al.*, 2001). In order to determine the total volume of water required to reduce the initial concentration of I_2 and NP to below

the applicable aquatic toxicity benchmarks (both acute toxicity and threshold for endocrine disruption potential in the case of NP), the total volume of iodine and NP released (mg) were divided by the applicable toxicity benchmarks (mg/L) to determine the required water volume (L).

2.3.1 Potential Environmental Concentrations and Potential for Toxicity

Potential concentrations in the marine environment in Atlantic Canada were calculated from a variety of sources based on either total use per facility, total use per fish, and total use per province (Parker, 2004; Muise and Associates, 2001; Roth, 1998). In order to derive a conservative estimation of the maximum expected environmental concentration, the highest volume identified in the literature was assumed to be discharged to the marine environment. A summary of the results is presented in Table 2.

Several assumptions regarding releases to the marine environment from salmon aquaculture facilities were made, including:

1) It was assumed that the Wescodyne and water solution would contain 100 mg/L of free iodine, and this concentration would be discharged directly to the marine environment. The United States Department of Agriculture (USDA) recommends a solution of Wescodyne containing approximately 100-200 mg of free iodine per litre of water for disinfecting human skin, clothing and boots, smooth surfaces, and aquaculture nets (USDA, 2002). According to the Alberta Agriculture, Food and Rural Development (AAFRD) (2000), 1 mL of full strength Wescodyne when mixed with 150 mL of clean water, makes a 100mg/L iodine solution. This solution will turn water dark brown (AAFRD, 2000). According to Parker (2004), when diluting Wescodyne with water to create a disinfection solution, aquaculture employees will pour enough Wescodyne into water to turn it brown (approximately 1:150 dilution or 100 mg/L free iodine). Therefore, during disinfection of boats and equipment, it is likely that the concentration of free iodine in the solution of Wescodyne and water would be 100 mg/L or greater, and following disinfection, this concentration would be released to the aquatic environment. In addition, given that 8.74% of the titratable iodine is in the form of nonylphenoxypoly(ethyleneoxy)ethanol-iodine, approximately 8.74 mg/L of this nonylphenolic compound would also be released to the aquatic environment as part of the Wescodyne solution.

2) The predicted concentrations of iodine and NP released to the environment were compared to the critical toxicity values of 0.99 mg/L for I_2 (Tooby *et. al.*, 1975) and 0.0039 mg/L for NP (Ward and Boeri, 1991) to determine whether unacceptable risks may occur as a result of Wescodyne to exposure.

3) The highest concentration of salmon aquaculture facilities in New Brunswick is in Passamaquoddy Bay, with 94 sites reported (Burridge, 2007). In order to be conservative in the assessment of potential exposure, it was assumed that all salmon aquaculture facilities in New Brunswick were situated in this Bay and all facilities were using similar volumes of Wescodyne on a regular basis. In Nova Scotia, a total of 40 salmonid aquaculture facilities were reported, with the greatest concentration of these sites identified in Yarmouth County, with (from west to east) six in Lobster Bay adjacent to Pubnico Harbour, and two in the outer Pubnico Harbour (Nova Scotia Department of Agriculture and Fisheries, 2007). It was assumed that this area contained the highest concentration of salmon aquaculture facilities in Nova Scotia and when calculating potential Wescodyne releases to the environment, it was assumed that all of these facilities were using similar volumes on a regular basis.

Calculation #1

According to Parker (2004), a maximum of 95 L of Wescodyne solution is expected to be used during any one application. Assuming the solution contains 100 mg/L free iodine, the total iodine released at any one time may be up to 9500 mg. In addition, given that 8.74% of the total iodine in Wescodyne is in

the form of nonylphenoxypoly(ethyleneoxy)ethanol-iodine (Cantox, 2001), up to 830 mg of this nonylphenolic compound would be released at any one time.

In order to dilute 95 L of Wescodyne solution containing 100 mg/L I_2 to less than 0.99 mg/L I_2, the maximum number of litres of sea water not impacted with Wescodyne required for sufficient dilution would be 9600 L. In order to dilute 95 L of Wescodyne containing approximately 8.74 g/L of nonylphenoxypoly(ethyleneoxy)ethanol-iodine (assumed to be of equal toxicity to NP) to less than 0.0039 mg/L NP, the total number of litres of sea water not impacted with Wescodyne required for sufficient dilution would be a maximum of 212,820 L.

Based on the dilution rates for six areas in the Lower Bay of Fundy according to Ernst *et. al.* (2001), the initial release concentration should be diluted between 1/200 and 1/3000 of the original concentration within five hours. Thus, with an initial concentration released of 100 mg/L I_2, the resultant concentration in the larger ambient environment surrounding the discharge would be expected to be between 0.5 and 0.033 mg/L within approximately five hours of release. In addition, the amount of NP present would range from 8.74 mg/L immediately following release, to between 0.04 mg/L and 0.003 mg/L within approximately five hours of release.

Calculation #2

As presented in Section 1.1 of this report, in 1999, the maximum identified gross amount of Wescodyne active ingredient (a.i.) of I_2 used in the marine environment in either Nova Scotia or New Brunswick was 21.3 g per tonne of salmon (based on information from six aquaculture facilities, as compared to 0.04 mg/tonne of fish for Steelhead trout [Muise and Associates, 2001]). According to Roth (1998), iodophor use in 1997 was 10,903 L in New Brunswick and 2590 L in Nova Scotia. No information was provided by Muise and Associates (2001) or Roth (1998) on use patterns (i.e. what quantities were used on cages vs. other equipment and/or clothing, dates of use, volumes released to the environment, locations of release, or concentrations measured in the aquatic environment either at the aquaculture sites, docks, or in the larger ambient aquatic environment).

According to Parker (2004), the majority of large-scale aquaculture operations occur in the marine environment. Therefore, in order to derive a potential concentration of Wescodyne available for exposure by organisms in the marine environment, the concentration of Wescodyne used per tonne of fish (based on Muise and Associates, 2001, see Table C.2 in Appendix C) was compared to the volume of fish production for both Nova Scotia (Nova Scotia Department of Agriculture and Fisheries, 2002) and New Brunswick (Parker, 2004). The main assumption with deriving a released concentration for 2002 was that the concentration of Wescodyne used per tonne of fish in 1999 would be similar to the concentrations used per tonne of fish in 2002.

In Nova Scotia, in 2002, approximately 2385 tonnes of fish were produced (primarily Atlantic salmon) (Nova Scotia Department of Agriculture and Fisheries, 2002), and in New Brunswick, approximately 40,000 tonnes of salmon were produced (Parker, 2004). Since approximately 21.3 g of Wescodyne active ingredient (iodine) was used per tonne of fish produced (Muise and Associates, 2001), for Nova Scotia, approximately 50,225 g (or ~50 kg) of that mixture was I_2, and for New Brunswick, 852 000 g (or 852 kg) of that mixture was I_2. Assuming that 100% of the Wescodyne used is released to the marine environment in equal amounts daily over 365 days, 138 g I_2 would be released daily to the marine environment in Nova Scotia, and 2,334 g I_2 would be released in New Brunswick. In addition to the iodine, the amount of nonylphenoxypoly(ethyleneoxy)ethanol-iodine released to the environment daily would be approximately 12,027 mg in Nova Scotia and 204,014 mg in New Brunswick.

For New Brunswick, assuming that all facilities are situated in Passamaquoddy Bay, the concentration of I_2 released to the marine environment would be approximately 2,300,000 mg/day, and the concentration on NP released would be approximately 204,000 mg/day. For Nova Scotia, assuming that the highest concentration of salmonid aquaculture facilities (eight) are located in the Pubnico area,

the total estimated daily release of I_2 in this area would be 27,521 mg/day, and the estimated release of NP would be 2405 mg/day.

In order to dilute these concentrations to below the critical toxicity values for I_2 and NP, approximately 2 million L of sea water would be required for I_2 and 52 million L of water would be required for NP in New Brunswick, and 28,000 L of sea water would be required for I_2, and approximately 600,000 L would be required for NP in Nova Scotia.

In the study conducted by Ernst *et. al.* (2001), within five hours of the release of a pesticide (cypermethrin) and a dye tracer (Rhodamine [WT] solution containing 20% rhodamine), at three locations in the Bay of Fundy, the mixture had dispersed between 479 and 1423 m in length, between 158 and 942 m in width, and between 1.6 and 11 m in depth, for a total impacted volume of between 985,928 and 6,032,097 m^3 ($9.86 \times 10^{+8}$ to $6.03 \times 10^{+9}$ L). Provided sufficient mixing were to occur, this volume of water would be sufficient to dilute the predicted concentrations to below the critical toxicity values within 5 hours of release at the edge of the plume. However, assuming that the concentration released would be approximately 100 mg/L I_2 and 8.74 mg/L NP, and a minimum dilution of 1/200 (Ernst *et. al.*, 2001), the resultant maximum concentrations within the highest concentrated area of the plume would be 0.5 mg/L I_2 and 0.0437 mg/L NP.

Calculation #3

Muise and Associates (1999) estimated that 122 kg of Wescodyne active ingredient (a.i.) (iodine) was used in New Brunswick in 1999 to produce 18,000 tonnes of salmon, and 1.428 kg of Wescodyne a.i. (iodine) was used in Nova Scotia to produce 1,785 tonnes of salmon. Latest available production figures for salmon show that New Brunswick produced 40,000 tonnes of salmon while Nova Scotia produced 2,385 tonnes of salmon in 2002. Based on the volumes of Wescodyne a.i. used in 1999 from Muise and Associates (1999), and salmon production figures from 2002, approximately 271.1 kg of Wescodyne active ingredient (iodine) was used in New Brunswick and 1.9 kg of Wescodyne active ingredient (iodine) was used in Nova Scotia in 2002.

Assuming that 100% of the Wescodyne used is released to the environment in equal amounts daily over 365 days, 743 g I_2 would be released to the marine environment in New Brunswick and 5.2 g I_2 released in Nova Scotia on a daily basis. In addition, 64,918 mg of NP would be released to the marine environment in New Brunswick on a daily basis and 457 mg of NP would be released on a daily basis in Nova Scotia. Assuming all New Brunswick salmon aquaculture sites are located in and around Passamaquoddy Bay, it was assumed that all Wescodyne released in New Brunswick would be into this area. For Nova Scotia, with the greatest concentration of salmonid aquaculture facilities located around Pubnico Harbour (eight facilities), the total estimated daily release of I_2 and NP in this area of Nova Scotia would be 1045 mg I_2 and 91 mg NP.

In order to dilute these concentrations to below the critical toxicity values for I_2 and NP, approximately 750,000 L of sea water would be required for I_2, and 16.6 million L of sea water would be required for NP in New Brunswick, and approximately 1000 L of sea water would be required for I_2, and 22,429 L of sea water would be required for NP in Nova Scotia.

Ernst *et. al.* (2001) estimated that at five hours following the release of a pesticide and dye tracer at three locations in the Bay of Fundy, the total impacted volume of water was between 985,928 and 6,032,097 m^3 ($9.86 \times 10^{+8}$ to $6.03 \times 10^{+9}$ L). Provided sufficient mixing were to occur, this volume of water would be sufficient to dilute the predicted concentrations to below the critical toxicity values within 5 hours of release.

Calculation #4

According to Alpha Chemicals (2006) and Roth (1998), the annual purchase of iodophor compounds per aquaculture facility ranged from 0 to 768 L. Assuming that all the facilities use 768 L per year of iodophors, that all iodophors used are Wescodyne, and that equal amounts are used daily over a 365 day period, the average daily quantity of Wescodyne used per facility would be 2.1 L/day. Assuming that the concentration of free iodine in the solution of Wescodyne and water used to disinfect boat and equipments is 100 mg/L or greater and 8.74% of that is nonylphenoxypoly(ethyleneoxy)ethanol-iodine, the total I_2 and NP released into the marine environment by each facility per day would be 210 mg and 18.35 mg, respectively. For New Brunswick, assuming all 94 aquaculture facilities are located in Passamaquoddy Bay, if each were to release Wescodyne to the marine environment on a daily basis, the total volume released could be as great as 197.4 L/day of Wescodyne, containing 19,740 mg I_2 and 1725 mg NP. In Nova Scotia, the greatest concentration of salmonid aquaculture facilities is in the Pubnico area (nine facilities), and if each of these were to release Wescodyne on a daily basis, the total volume released could be as great as 18.9 L of Wescodyne, containing 1890 mg I_2 and 166 mg NP.

In order to dilute these concentrations to below the critical toxicity values for I_2 and NP, approximately 20,000 L/day of sea water would be required for I_2, and approximately 440,000 L/day for NP in New Brunswick, and approximately 1900 L/day of seawater would be required for I_2, and approximately 42,000 L/day for NP in Nova Scotia.

Based on the study conducted by Ernst *et. al.* (2001), five hours following the release of a pesticide and dye tracer at three locations in the Bay of Fundy, the total impacted volume was between 985,928 and 6,032,097 m^3 ($9.86 \times 10^{+8}$ to $6.03 \times 10^{+9}$ L). Provided sufficient mixing were to occur, this volume of water would be sufficient to dilute the predicted concentrations to below the critical toxicity values within 5 hours of release at the edge of the plume. However, assuming that the concentration released would be approximately 100 mg/L I_2 and 8.74 mg/L NP, and a minimum dilution of 1/200 (Ernst *et. al.*, 2001), the resultant maximum concentrations within the highest concentrated area of the plume would be 0.5 mg/L I_2 and 0.0437 mg/L NP.

Table 2: Calculated Potential Wescodyne Concentrations in the Marine Environment Following Release

Calc. #	Location	Amount of Wescodyne Used (L)	Concentration of I_2 released (mg)	Concentration of NP released (mg)	Critical Toxicity Value (CTV) (mg/L)	Dilution Required for Reduction of I_2 to Below CTV (L)	Dilution Required for Reduction of NP to Below CTV (L)	Maximum Expected Dilution (over 5 hours) based on Ernst *et. al.* (2001) (L)
1	Any facility in NB or NS at any one time	95	9,500	830	0.99 (I_2) 0.0039 (NP)	9,596	212,897	$9.86 \times 10^{+8}$ to $6.03 \times 10^{+9}$
2	Passamaquoddy Bay, NB	Not identified	2,334,247	204,013	0.99 (I_2) 0.0039 (NP)	2,357,825	52,311,064	$9.86 \times 10^{+8}$ to $6.03 \times 10^{+9}$
	Pubnico, NS	Not identified	27,521	2,405	0.99 (I_2) 0.0039 (NP)	27,799	616,747	$9.86 \times 10^{+8}$ to $6.03 \times 10^{+9}$
3	Passamaquoddy Bay, NB	Not identified	742,770	64,918	0.99 (I_2) 0.0039 (NP)	750,273	16,645,670	$9.86 \times 10^{+8}$ to $6.03 \times 10^{+9}$
	Pubnico, NS	Not identified	1,045	91	0.99 (I_2) 0.0039 (NP)	1,056	23,429	$9.86 \times 10^{+8}$ to $6.03 \times 10^{+9}$
4	Passamaquoddy Bay, NB	197.4	19,740	1,725	0.99 (I_2) 0.0039 (NP)	19,978	443,244	$9.86 \times 10^{+8}$ to $6.03 \times 10^{+9}$
	Pubnico, NS	16.8	1,683	147	0.99 (I_2) 0.0039 (NP)	1,700	37,723	$9.86 \times 10^{+8}$ to $6.03 \times 10^{+9}$

Note: The Ernst *et. al.* (2001) study assessed the dispersion of cypermethrin at three locations in the Lower Bay of Fundy, New Brunswick, in 1997, with detectable dye concentrations identified up to 2 to 5.5 hours following release with distances travelled ranging from 900 to 3000 m and dye concentrations generally 1/1000 to 1/2000 the pre-release concentrations.

2.3.2 Assessment of Toxicity

Based on the environmental concentrations calculated in Section 3.2.1 and presented in Table 2, the volume of water required to dilute Wescodyne to acceptable concentrations range between approximately 20,000 L and 597,000 L in Nova Scotia, and between 440,000 and 51 million L in New Brunswick.

Based on dilution factors from the Lower Bay of Fundy (between 1/200 and 1/3000 of original release concentration [Ernst *et. al.*, 2001]), and assuming the Wescodyne solution released contained 100 mg/L I_2, within 5 hours of release, I_2 concentrations would range from 0.5 mg/L to 0.033 mg/L, and NP concentrations (as nonylphenoxypoly(ethyleneoxy)ethanol-iodine) would range from 0.04 to 0.003 mg/L. The critical toxicity value for I_2 was determined to be 0.99 mg/L (based on an extrapolated three-month LC_{50} of 9.9 mg/L [Tooby *et. al.*, 1975] for the Harlequin fish and applying a factor of 10 to convert an LC_{50} to a NOEC). Thus, the potential environmental concentrations based on the calculations identified in Section 2.3.1, would indicate that there should be no long-term unacceptable risks to aquatic receptors from Wescodyne releases to the marine environment from aquaculture facilities. The critical toxicity value selected for NP was the lowest NOEC identified in the literature of 0.0039 mg/L (Ward and Boeri, 1991). With a dilution of 1/200, the NP concentration in the most concentrated area of the plume may exceed this critical toxicity value by an order of magnitude.

Therefore, depending on the concentration released to the local aquatic environment, the number of facilities using Wescodyne at any given time, the dilution rate into the larger environment, and the expected exposure duration (as a percentage of lifetime exposure), NP concentrations at and/or near the discharge point may exceed critical toxicity values (including for endocrine disruption) and may result in adverse effects to nearby aquatic organisms.

2.4 International Activities

Based on a review of the literature, Scotland, Wales, and the United States use Wescodyne in the aquaculture industry to disinfect eyed fish eggs, particularly against ISA, and/or also for the disinfection of aquaculture equipment and worker clothing (FRS Marine Laboratory, 1999; Department for Environment, Food and Rural Affairs, National Assembly for Wales, 2002; USDA, 2002). Each country has defined acceptable concentrations and locations for disinfection of fish eggs and aquaculture equipment, and for disposal of waste product. Scotland has produced a Code of Practice in order to minimize the impacts of ISA, and as part of that document, have produced procedures for disinfection at aquaculture facilities. This document indicates that discharge of disinfectants to the environment is controlled under the Food and Environmental Protection Act 1985 Part II Deposits in the Sea, and a discharge consent issued by the Scottish Environment Protection Agency must be obtained for discharges made during activities such as net and cage disinfection, both at cage site and shore bases (FRS Marine Laboratory, 1999). In addition, Scotland keeps records quantities of anti-foulants, anaesthetics, anti-microbials, anti-parasitics, and disinfectants (including iodine and iodophor) used at individual aquaculture facilities in Scotland each year (SEPA, 2007). Iodophors are the most common disinfectants used at these facilities, with 3422 L used at 30 facilities in 2005 (21% of the total volume of all 26 disinfectants reported to having been used).

The documents from Wales and the U.S. provide instructions on procedures to be used for disinfection using iodophors at aquaculture facilities, however, they are not formally defined as Guidelines. The Wales guidance indicates that all Wescodyne solutions and washings should preferably be disposed of into a sewer, but where this is not possible, they can be safely released very slowly into fast moving waters (Department for Environment, Food and Rural Affairs, National Assembly for Wales, 2002). No mention is made in the US document of where disinfection should occur or the appropriate method/location for disposal of waste product (USDA, 2002).

3.0 CONCLUSIONS

Based on an understanding of the physical, chemical and toxicological properties of iodine, NP, and where available, Wescodyne, it appears that components of the iodophor compound are moderately persistent in water and sediment (primarily the NP component) but not expected to bioaccumulate in aquatic organisms.

Wescodyne, based on preliminary calculations using limited data, may be entering the environment in concentrations that may pose unacceptable risks to marine aquatic receptors in Atlantic Canada, depending on the concentration released to the local aquatic environment, the number of facilities using Wescodyne at any given time, and the dilution rate into the larger environment.

3.1 Sources of Uncertainty

There are several sources of uncertainty associated with this risk assessment of Wescodyne. These are identified below.

1) No toxicity studies were conducted using Wescodyne or iodine on marine species in marine environments (i.e. even salmon, an anadramous species, was assessed in fresh water);

2) Actual volumes of Wescodyne, free iodine, and nonylphenoxypoly(ethyleneoxy)ethanol-iodine released to the marine and fresh water environments in Atlantic Canada are not known;

3) Other iodophor compounds may also be in use at aquaculture facilities in Atlantic Canada (instead of or in addition to Wescodyne), which may result greater releases of iodine to the ambient aquatic environment than presented in the calculations in Section 2.3.1;

3) Very little physical, chemical or toxicological information exists for Wescodyne or nonylphenoxypoly(ethyleneoxy)ethanol-iodine, thus in order to assess the environmental fate of Wescodyne it was necessary to use the experimental and/or predicted data from iodine (the active ingredient of Wescodyne) and nonylphenol as a surrogate compound to represent the NP compound in Wescodyne. Nonylphenol was used as a surrogate compound because it was considered to be representative of NPs in terms of its persistence, bioaccumulation potential and toxicity and is expected to be more toxic to aquatic receptors than NPEs with longer ethoxylate chain lengths, and nonylphenoxypoly(ethyleneoxy)ethanol-iodine in particular (Hellyer, 1991);

5) Wescodyne concentrations in the aquatic environment in Atlantic Canada have not been measured (either at aquaculture sites or in the ambient environment);

6) Exposure concentrations were based on Wescodyne use per tonne of fish from Muise and Associates (2001) for 1999 and fish production data from Nova Scotia and New Brunswick for 2002, and do not necessary reflect actual Wescodyne concentrations used for 2002;

7) One exposure concentration calculation assumed daily Wescodyne releases of approximately the same volume per day, which may not represent actual release patterns; and

8) Exposure concentrations assumed that all Wescodyne used would be released from one localized area as opposed to reality where releases are expected to occur at different locations at different times.

9) No information was identified on expected dilution rates in the Bay of Fundy over a period of time greater than 5 hours. Thus, no potential concentrations available for longer term exposure could be derived. Critical toxicity values for Wescodyne, iodine and NP were based on the most sensitive toxicity study identified in the literature. Given that some of these studies were for longer exposure durations (or for endpoints other than mortality), the values used in this report may over-estimate the risk given the expected exposure duration (pulse/intermittent vs. continual) at aquaculture facilities in Atlantic Canada.

4.0 RECOMMENDATIONS

Based on the analysis contained herein, the following is recommended in order to reduce the amount of uncertainty in this assessment:

1) The concentrations of iodine and surfactant should be determined in the environment (including sediment and surface water) after operational uses of Wescodyne. Samples should be collected immediately following release, one hour following release, and five hours following release at the source and also down-current of the source, at various distances to be determined at the time of the field program development. In addition, surface water should be collected at an appropriate reference site, such as a basin with no aquaculture activities and no impacts expected from current or historical aquaculture activities (i.e. not a basin that may be inundated with waste products from an aquaculture facility in an adjacent or nearby basin due to tidal and current action). These samples should also be analysed for iodine to determine typical concentrations in the local marine environment.

2) The aquaculture industry, industry groups, Wescodyne manufacturers, and/or Wescodyne distributors should be contacted (either formally or informally) to determine the quantities and use patterns of Wescodyne used at aquaculture sites in Atlantic Canada;

3) Acute and chronic toxicity studies using salt water species should be conducted to determine the effect of elevated concentrations of Wescodyne on marine species; and

4) Endocrine disruption potential should be assessed for Wescodyne based on the concentrations released to the aquatic environment due to the presence of a NP compound which is a known endocrine disruptor.

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APPENDIX A SEARCH STRATEGIES EMPLOYED

Data relevant to the environmental screening assessment of Wescodyne were identified in original literature, review documents, commercial and government databases and indices. This included on-line searches conducted during December 2003 and December 2007 using CAS registry numbers from the following databases:

ASFA (Aquatic Sciences and Fisheries Abstract), CESARS (Chemical Evaluation Search and Retrieval System, Ontario Ministry of the Environment and Michigan Department of National Resources; 2003), CHEMINFO (Canadian Centre for Occupational Health and Safety; 2003), CHEMFATE (2003), ECOTOX (Ecotoxicology, U.S. Environmental Protection Agency; 2007), National Library of Medicine (2007) which includes HSDB (Hazardous Substances Data Bank) and Toxline, Oceanic Abstracts, Medline (1998-2003), MSDS (Material Safety Data Sheets, Canadian Centre for Occupational Health and Safety; 2003), and SciFinder.

In addition to retrieving the references from the literature database search, ongoing scans were conducted of the open literature, conference proceedings and the internet for relevant information. Data obtained to January 2004 were considered initially in this technical draft document, with an update in December, 2007, to determine the existence of any new and relevant literature.

APPENDIX B. SUMMARY OF TOXICOLOGY STUDIES IDENTIFIED

Table B.1: Summary of Toxicology Studies Identified for Wescodyne, Iodine and Nonylphenol

Species	Test Duration	Effect Level (mg/L)	Study Details	Reference
Wescodyne				
Salmo salar Atlantic Salmon	10 minutes	• No effect value = 75 (free iodine)	• Fresh water • Static test	Alderman, 1984
Micropterus salmonides Largemouth bass eggs	15 minutes	• LC100 = 200 mg/L (free iodine)	• Fresh water • Static test	Wright and Snow, 1975
Oncorhynchus mykiss Rainbow trout	15 minutes	• LC_{50} = 118 at pH 3.1 – 700 at pH 7.0 • LC_{50} in waters of varying hardness and pH 6.9 (20-320 ppm CaCO3) = 538-562	• Fresh water • Static test	Amend, 1974
Oncorhynchus mykiss Rainbow trout	24 hours 48 hours 96 hours 3 months	• LC_{10} = 17.6 at pH 6.1 • LC_{50} = 29.9 at pH 6.1 • LC_{10} = 13.5 at pH 6.1 • LC_{50} = 23.4 at pH 6.1 • LC_{10} = not identified • LC_{50} = 22.8 at pH 6.1 • LC_{50} = 19.9 at pH 6.1 (all free iodine)	• Iodine • Fresh water • Flow-through test	Tooby *et. al.*, 1975
Rasbora heteromorphos Harlequinfish	24 hours 48 hours 96 hours 3 months	Non-ionic surfactant • LC_{10} = 15.8 at pH 7.0 - 47.4 at pH 8.1 • LC_{50} = 21.1 at pH 7.0 - 64.4at pH 8.1 • LC_{10} = 9.9 at pH 7.0 – 35.1 at pH 8.1 • LC_{50} = 16.4 at pH 7.0 - 58.5 at pH 8.1 • LC_{10} = 9.9 at pH 7.0 – 21.1 at pH 8.0 • LC_{50} = 15.2 at pH 7.0 - 52.7 at pH 8.1 • LC_{50} = 9.9 at pH 7.0 to 29.8 at pH 8.1	• Fresh water • Flow-through test	Tooby *et. al.*, 1975
Oncorhynchus mykiss Rainbow trout	35 days	• Mortality endpoint = 100	• Fresh water • Static test	Amend, 1974
Iodine				
Poecilia reticulate Guppy	24 hours	• LC_{50} = 3.0	• Fresh water • Static test	Yarzhombek, *et. al.*, 1991
Rasbora heteromorphos Harlequinfish	24 hours 48 hours 96 hours 3 months	• LC_{50} = 29.9 at pH 6.1 • LC_{50} = 23.4 at pH 6.1 • LC_{50} = 22.8 at pH 6.1 • LC_{50} = 19.9 at pH 6.1	• Fresh water • Flow-through test	Tooby *et. al.*, 1975
Daphnia magna	96 hours	• LC_{50} = 0.59	• Fresh water • Static test	Laverock *et. al.*, 1995
Ictalurus punctatus Channel catfish	96 hours	• LC_{50} = 0.44	• Fresh water	LeValley, 1982
Oncorhynchus mykiss Rainbow trout	96 hours	• LC_{50} = 1.67	• Fresh water • Static test	Laverock *et. al.*, 1995
Oncorhynchus mykiss Rainbow trout	14 day	• Mortality in hatching = 300	• Fresh water • Pulse test	Marking, *et. al.*, 1994

Table B.1: Summary of Toxicology Studies Identified for Wescodyne, Iodine and Nonylphenol

Species	Test Duration	Effect Level (mg/L)	Study Details	Reference
Nonylphenol				
Daphnia magna	48 hours	• EC50 (immobilization) = 0.19	• Fresh water	Comber *et. al.*, 1993
Oncorhynchus mykiss Rainbow trout	72 hours	• LC50 = 0.194 • EC50 (estrogenic concentration) = 0.0144	• Fresh water • Flow-through test	Lech *et al.*, 1996
Oryzias latipes Japanese medaka	72 hours	• LC50 (embryo) = 0.13 • LC50 (male/female) = 0.85/0.87	• Fresh water • Daily renewal	Kashiwada *et al.*, 2002
Selenastrum capricornutum Green algae	96 hours	• NOEC = 0.092	• fresh water	Weeks *et. al.*, 1996
Hyalella azteca	96 hours	• LC50 = 0.0207	• Fresh water	Brooke, 1993
Eohaustorius estuarius Amphipod	96 hours	• LC50 = 0.227 43.7% NP lost from beaker over 96 hrs.	• Saltwater • Static	Hecht and Boese, 2002
Americamysis bahia Opossum shrimp	96 hours	• LC50 = 0.0606	• Saltwater • Flow-through test	Lussier *et. al.*, 2000
Palaemonetes vulgaris Marshgrass shrimp	96 hours	• LC50 = 0.0594	• Saltwater • Flow-through test	Lussier *et. al.*, 2000
Homarus americanus American lobster	96 hours	• LC50 = 0.071	• Saltwater • Static	Lussier *et. al.*, 2000
Dyspanopeus sayi Say mud clam	96 hours	• LC50 > 0.195	• Saltwater • Flow-through test	Lussier *et. al.*, 2000
Mulinia lateralis Clam	96 hours	• LC50 = 0.0379	• Saltwater • Static	Lussier *et. al.*, 2000
Oncorhynchus mykiss Rainbow trout	96 hours	• LC50 = 0.19	• Fresh water	Dwyer *et. al.*, 1995
Pimephales promelas Fathead minnow	96 hours	• LC50 = 0.128	• Fresh water	Brooke, 1993
Cyprinodon variegatus Sheepshead minnow	96 hours	• LC50 = 0.31	• Saltwater	Ward and Boeri, 1990
Cyprinodon variegatus Sheepshead minnow	96 hours	• LC50 = 0.142	• Saltwater • Flow-through test	Lussier *et. al.*, 2000
Pleuronectes americanus Winter flounder	96 hours	• LC50 = 0.017	• Saltwater	Lussier *et. al.*, 1996
Menidia beryllina	96 hours	• LC50 = 0.070	• Saltwater	Lussier *et.*

Table B.1: Summary of Toxicology Studies Identified for Wescodyne, Iodine and Nonylphenol

Species	Test Duration	Effect Level (mg/L)	Study Details	Reference
Inland silverside			• Flow-through test	*al.*, 2000
Fundulus heteroclitus Killifish	96 hours	• LC50 = 0.204	• Saltwater • Daily renewal	Kelly and Di Giulio, 2000
Oncorhynchus mykiss Rainbow trout	14 day	• LOEC (reproduction) = 0.0061	• Fresh water • Flow-through test	Thorpe *et al.*, 2001
Daphnia magna	21 day	• LC50 = 0.1	• Fresh water	Comber *et. al.*, 1993
Mysidopsis bahia Mysid shrimp	28 day	• NOEC = 0.0067	• Salt water • Survival and reproductive effects	Ward and Boeri, 1991
Mysid shrimp	29 day	• NOEC (length) = 0.0039	• Saltwater	Weeks *et. al.*, 1996
Oncorhynchus mykiss Rainbow trout	91 day	• NOEC = 0.006	• Fresh water • survival endpoint	Brooke, 1993

Table B.2: Disinfection Capability of Various Free Iodine Concentrations

Test Organism	Concentration	Duration of Exposure	Conclusion and Additional Information	Reference
Trout eggs	50 mg/L iodine	10 minutes	• Recommended solution to disinfect fertilized trout ova from organisms of furunnculosis adhering to the outside of fish eggs • May be highly toxic to unfertilized ova and live fish at this concentration	Department for Environment, Food and rural Affairs, National Assembly for Wales, 2002
Brook trout eggs	100 mg/L iodine as Wescodyne	Not stated	• At 100 mg/L, egg transmission of IPN occurred in both treated and non-treated eggs	Bullock *et. al.*, 1976
Rainbow trout eggs	50-100 mg/L iodine	15 minute dip	• Effective to disinfect eggs provided pH is maintained above 6.0, water has low organic content, and rinsing eggs with clean water following disinfection	Amend, 1974
Trout and salmon eggs	75-100 mg/L iodine	10 minutes	To control surface bacteria and viruses without effects to trout or salmon eggs	AAFRD, 2000
Eyed salmon and trout eggs	100 mg/L available iodine	10 minutes	• 0% mortality • No significant mortality provided pH is with a reasonable level	Alderman, 1984
Dark eyed eggs	50 mg/L (not stated whether Wescodyne or iodine)	10 minutes	• Recommended concentration for disinfection • Eggs should be rinsed before and three times after treatment	Gerard, 1972

Table B.2: Disinfection Capability of Various Free Iodine Concentrations

Test Organism	Concentration	Duration of Exposure	Conclusion and Additional Information	Reference
Eyed fish eggs	100 mg/L iodine	30 minutes	• Recommended concentration in order to disinfect eyed fish eggs • Eggs should be rinsed with water before and after fertilization and should be between pH 6-8	USDA, 2002
Gametes during fertilization	25 mg/L iodine	Several hours	• Recommended concentration to disinfect gametes • Eggs should be rinsed with water before and after fertilization and should be between pH 6-8	USDA, 2002
Largemouth bass eggs	100-150 mg/L free iodine	15 minutes	• 88% hatch rate • At 100 mg/L Wescodyne, bacteria recovered following treatment • At 150 mg/L, only one of six experiments had bacteria recovery following treatment	Wright and Snow, 1975
Largemouth bass eggs	200 mg/L iodine	15 minutes	• 0% hatch rate	Wright and Snow, 1975
American lobsters	250, 300, 500 and 1000 mg/L Wescodyne (4, 4.8, 8, and 16 mg/L iodine)	5 treatments at 15 minutes each	• To eliminate ciliate infestation • None of treatments eliminated ciliate populations • Abrupt drop in infection levels and overall retardation of infestation was identified among lobsters	Boghen, 1982
IPN virus	25 mg/L iodine 12 mg/L iodine	25 seconds 30 seconds	• Destroyed infectious pancreatic necrosis (IPN)	Amend and Pietsch, 1972
IHN virus	25 mg/L iodine 12 mg/L iodine 27 mg/L iodine	15 seconds 30 seconds 15 minutes	• Effectively destroyed infectious hematopoietic necrosis (IHN) virus	Amend and Pietsch, 1972
IHN, IPN, and VHS viruses	16 mg/L iodine	5 minutes	• Effectively destroyed infectious hematopoietic necrosis (IHN), IPN, and viral hemorrhagic septicaemia virus (VHS)	Amend and Pietsch, 1972
E. coli	25 mg/L iodine	15 seconds	100% mortality	Bogash, 1955
Myco-bacterium tuberculosis	50 mg/L iodine	60 seconds	100% mortality	Bogash, 1955
Human skin, smooth surfaces, nets, boots, clothing	Approximately 200 mg/L iodine	Several seconds	Approved in U.S. for destruction of bacteria and viruses on human skin, smooth surfaces, clothing and boots, and aquaculture nets	USDA, 2002

APPENDIX C. NOVA SCOTIA AND NEW BRUNSWICK USE PATTERN INFORMATION

Tables C.1 and C.2 do not represent the production from three farms but from the combined production and material consumption data from three associations of fresh water and marine farms.

Table C.1: Gross Quantities of Chemicals Used in 1999 for New Brunswick and Nova Scotia by Salmon Producers in Fresh water (Muise and Associates, 2001)

Quantity per 780,000 Fish[1]	A.I. per 1000 Fish[1]	Quantity per 1,067,000 Fish[2]	A.I. per 1000 Fish[2]	Quantity per 400,000 Fish[3]	A.I. Per 1000 Fish[3]
Wescodne					
0	0	250 L bulk	3.51 g	108 L bulk	4.1 g
Mikroklene (Iodine)					
200 L	3.85 g	N/a	N/a	N/a	N/a

Notes: A.I. = active ingredient
N/a = not available
1 – represents reported figures from 2 fresh water facilities – smolt distributed to up to 35 marine farms
2 – represents reported figures from 3 fresh water facilities – smolt distributed to up to 22 marine farms
3 – represents reported figures from 2 hatcheries – smolt distributed to up to six marine farms

Table C.2: Gross Quantities of Chemicals Used in 1999 for New Brunswick and Nova Scotia in the Marine Environment (Muise and Associates, 2001)

Quantity per 3500 Tonnes[1]	A.I. per Tonne[1]	Quantity per 4,800 Tonnes[2]	A.I. per Tonne[2]	Quantity per 1,800 Tonnes[3]	A.I. Per Tonne[3]
Wescodyne					
N/a	N/a	N/a	N/a	2400 L bulk	21.3 g
Mikroklene (Iodine)					
1116 L	5 g	N/a	N/a	0	0

Notes: A.I. = active ingredient
N/a = not available
1 - represents reported figures from up to 35 marine farm sites
2 – represents reported figures from up to 22 marine farm sites
3 – represents reported figures from up to six marine farm sites

Table C.3 presents an estimate of gross chemical use by province for the year 1999. Column 2 lists the quantities of chemicals related to a total of 2.25 million fish in fresh water and 5300 tonnes of market fish from marine production. From these quantities, chemical use associated with gross production figures were calculated for New Brunswick and Nova Scotia. Annual production figures for farmed salmon for 1999 were obtained from the New Brunswick Department of Fisheries and Aquaculture and the Nova Scotia Department of Fisheries and Aquaculture (Muise and Associates, 2001).

Table C.3: Estimate of Chemical Use by Province for Salmon Production in 1999 (Muise and Associates, 2001)

Trade Name	NB A.I. per 2.25 Million Fish	NB Est. Total Use (4.0 Million Fish)[1]	NS A.I. per 200,000 Fish	N.S. Est. Total Use (397,000 Fish)[2]
Fresh water				
Wescodyne	5.37 g	9.6 g	0.82 g	1.6 g
Mikroklene	3 g	5.3 g	0	0
Trade Name	**NB A.I. per 5300 Tonnes**	**NB Est. Total Use (18,000 Tonnes)**	**NS A.I. per 900 Tonnes**	**N.S. Est. Total Use (1785 Tonnes)**
Marine				
Wescodyne	36 kg	122 kg	720 g	1.428 kg
Mikroklene	5 g	17 g	0	0
Notes: 1 – estimated based on column 2 2 – estimated based on column 4				

Table C.4: Estimate of Wescodyne Use for Steelhead Trout Production in NS in 1999 (Muise and Associates, 2001)

Quantity per 187,000 Fish	A.I. per 1000 fish	Quantity per 1,980,000 Fish	A.I. per 1000 Fish
Fresh water			
4 L	0.32 g	8 L	0.06 g
Quantity per 425 Tonnes	**A.I. per Tonne**	**Quantity per 4,500 Tonnes**	**A.I. per Tonne**
Marine			
2 L	0.07 g	12 L (4 L x 6 sites)	0.04 g

Table C.5: Estimate of Wescodyne Use by Province for Steelhead Trout Production in 1999 (Muise, and Associates, 2001)

A.I. per 2.17 Million Fish	Total Quantity NF (741,800 Fish)	Total Quantity NS (1,450,000 Fish)	Total Quantity NB (242,000 Fish)	Total Quantity PEI (273,500 Fish @ 300 g)
Fresh water				
180 g	61.5 g	120 g	20 g	23 g
A.I. per 4925 Tonnes	**Total Quantity NF (2003 Tonnes)**	**Total Quantity NS (3924 Tonnes)**	**Total Quantity NB (550 Tonnes)**	**Total Quantity PEI (Not applicable)**
Marine				
210 g	85.4 g	167 g	24 g	n/a